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Estrogen receptor alpha (ERα) is the most common target of treatment in human breast cancer. The expression of ERα has long been known as a positive prognostic indicator. While human breast cancers are composed of heterogeneous populations of cells, previous studies of ERα expression and function have focused on bulk tumor cells. With the recent discovery that breast cancer growth is driven by a tumorigenic subpoputlation of cancer cells, it has become vital to study the role of ERα in this population of cells.

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INTRODUCTION

Human breast cancers can be divided into two main groups: estrogen receptor alpha (ERα) positive and ERα negative tumors. It has long been known that expression of ERα within a tumor is a positive prognostic indicator. While cancer research has achieved great progress in identifying the molecular and biochemical changes that are involved in cancer formation, the effects of these changes on different cell populations within a tumor have remained elusive. Recent evidence has indicated that a minority subpopulation of tumorigenic cells within a breast tumor is the only population possessing the abilities of self-renewal and indefinite proliferation. This population gives rise to both more tumorigenic cells and the non-tumorigenic cells which form the bulk of the tumor. Since previous studies investigating the action of ERα have focused on bulk tumor samples without regard to tumorigenic status, it is vital to study the action of ERα in tumorigenic breast cancer cells. In the first year of this grant, we have made progress in the goals of the application. A summary of the progress in the tasks follows.

BODY

Task 1. To establish a xenograft tumor library of at least ten tumors from which the tumorigenic cell population has been positively identified, and determine their response to anti-estrogen therapy. Obtaining needle biopsy samples from patients that have not been exposed to anti-estrogen therapy has proved more difficult than first anticipated. While efforts on this front continue, we have moved forward with the tumors we currently have, which have been exposed to anti-estrogen therapy in the past. Untreated tumors will be used as they become available. We currently have positively identified the tumorigenic population from 10 tumors, an increase from only 7 last year. Additionally, we have confirmed that we can reliably sort the tumorigenic population for at least five passages in mice. Since we were hoping to obtain untreated tumors, we are just beginning to test our current tumors for response to tamoxifen.

Task 2. To determine the expression profile and subcellular localization of ERα in the tumorigenic and non-tumorigenic cell populations from each tumor. Several antibodies were screened before one was identified that was reliable for our purposes. To date, we have screened three of the tumors for the expression and localization of ERα in both the tumorigenic and non-tumorigenic populations. Figure 1 shows data from one of the patients. All three tumors examined had been identified as ERα-positive in the clinic. Our data indicated that both the tumorigenic and non-tumorigenic cell populations were ERα-positive in each tumor.

Tumor 1

Non-Tumorigenic

Tumorigenic

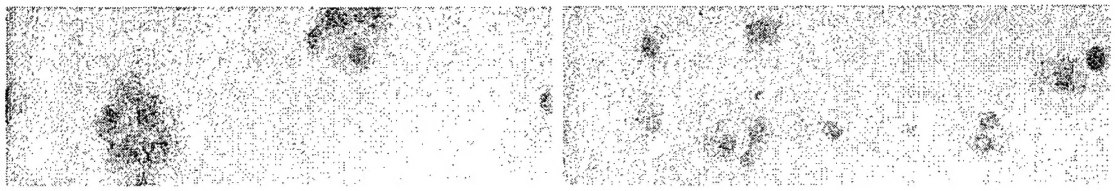

Figure 1. Cells sorted by flow cytometry were resuspended in HBSS with 6% heat-inactivated calf serum and cytospun onto coverslips at 1000 rpm for 6 minutes. The cells were then fixed in 4% paraformaldehyde for 20 minutes at room temperature. After washing with PBS three times, immunohistochemistry was performed using the Histostain Plus kit from Zymed and visualizing using the DAB substrate. Briefly, cells were blocked with goat serum (Zymed) for 10 minutes Before 1.5 hour incubation at room temperature with the primary antibody (rabbit polyclonal anti-estrogen receptor clone 18-0174 from Zymed). After washing three times with PBS, the secondary antibody incubation was performed at room temperature for one hour. After washing three times with PBS, the DAB substrate with enhancer (Zymed) was used according to the manufacturer's instructions. Counterstaining with hematoxylin (Zymed) was performed for 45 seconds. The coverslips were then washed in PBS three times and mounted In GVA mounting media (Zymed). Note that the nuclei of both populations of cells stain positively for ER.

Task 3. To explore the impact of ERα action on the growth and biology of tumorigenic and non-tumorigenic cells in vitro by measuring cell growth and activation of downstream effectors. While this task is scheduled for the following year, we have begun developing the reagents necessary to perform the experiements. We have identified an appropriate antibody for epidermal growth factor receptor; several have been tested for activated mitogen-activated protein kinase (MAPK) and activated Akt. The MAPK and AKT antibodies tested have been unsatisfactory so we are screening more as they become available.

Task 4. To obtain samples of tumor cells treated during xenograft with either extrogen, tamoxifen, fulvestrant, or buffer and looking for changes in tumorigenic cell distribution, downstream effector activation, or clonogenicity. These experiments depend upon further progress on Task 1. Cells will be harvested when the tumors are tested for response to estrogen therapy so analysis can begin.

KEY RESEARCH ACCOMPLISHMENTS

-Established protocol to stain for ERα on cytospun samples.
-Characterized tumorigenic population in multiple tumors.

REPORTABLE OUTCOMES

-"Expression of Estrogen Receptor Alpha in Tumorigenic and Non-Tumorigenic Human Breast Cancer Cells." Poster, University of Michigan Cellular and Molecular Biology Program Annual Symposium 2003.

REFERENCES

None

APPENDICES

None